STUDY OF ROLE OF FNAC AND IMPRINT CYTOLOGY IN THE DIAGNOSIS OF CERVICAL LYMPHADENOPATHY

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **" A STUDY OF ROLE OF FNAC AND IMPRINT CYTOLOGY IN THE DIAGNOSIS OF CERVICAL LYMPHADENOPATHY"** submitted by **Dr. G. RAVIKUMAR** to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of **M.S Degree Branch – I (General Surgery)** is a bonafide research work were carried out by him under direct supervision & guidance.

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DECLARATION

I **Dr. G. RAVIKUMAR** declare that, I carried out this work on, **"A STUDY OF ROLE OF FNAC AND IMPRINT CYTOLOGY IN THE DIAGNOSIS OF CERVICAL LYMPHADENOPATHY"** at the Department of Surgery, Govt. Rajaji Hospital during the period of June 2006 to October 2008. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, diploma to any other University, Board either in India or abroad.

This is submitted to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.S degree examination in General Surgery.

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INTRODUCTION

In 1966 bamforth defined tissue cytology as the examination of cells obtained by needle or drill biopsy in solid organ, or tissue masses or smear from the cut surface of such materials, freshly removed by surgical biopsy.

Diagnostic methods are in constant evoluation. New procedures which can be shown to be accurate and reliable gradually become established. Replacing those of lesser value. FNAC provides an extremely high true positive rate of diagnostic and yet relies heavily on the clinician to sample an appropriate area, properly.

The clinical diagnosis of lymphadenopathy is of great importance, as clinical assessment may be wildly erroneous. For the general surgeon as well specialist disciplines enlarged lymph nodes are a constant worry and FNAC if of value to reduce delay in identification of diagnosis.

Cytomorphological studies contemporarily are accepted as reliable safe and rapid procedures for a wide variety of lesions

and observation by various workers unequivocally have demonstrated that reliability of cytodiagnosis is as much as that of conventional HPE and there exists a remarkable correlation between the two methods in arriving at the diagnosis.

Cytomorphology incorporates FNAC & imprint cytology and though (both involves the study of cellular morphology) the harvest reaped in both are cells, there is one substantial difference between the two former is a closed procedure as compared to the latter which is possible only when the lesion is open.

At first glance, imprint cytology appears redundant since open biopsy yields tissue for HPE study. However careful scrutiny reveals certain distinctive advantages of imprint cytology study. Due to excellent cellular details, simplicity speed and sufficient cellular yield with preservation to some extent of histological pattern of imprint tissues, imprint cytology not only forms an adjuvant to HPE, it has tremendous positive implication in frozen section, if not superior to it.

Thus once a FNAC report makes it mandatory to opt for an open biopsy, Imprint Cytology forms a major corroborator not only for an intraoperative diagnosis but also during routine post operative histopathology reporting.

FNAC & Imprint Cytology form the rapid tools in the diagnostic armamentorium of surgeons and pathologists.

AIMS AND OBJECTIVES

1. To assess the role of FNAC and Imprint cytology in the diagnosis of cervical lymphadenopathy.
2. To correlate the findings of FNAC and Imprint Cytology with final HPE report.

EVOULATION OF CYTOLOGY

Imprint cytology :

As the knowledge about cancer pathology and treatment increased there came an awareness and need for rapid diagnosis of surgically removed specimens. In a pioneering study by Dudgeon and Patrick published in 1927, anesthesia new procedure for rapid diagnosis of surgically removed specimens by cytology, ‘wet film’ method was described and its use was judged highly accurate after comparison with routine paraffin section of the same material (Dudgeon L.S. and Patrick C.V., 1927)6,7 scrapping technique of dudgeon and Patrick has been revived and modified as “SCRIMP” (scrap/imprint) technique. This claims to be highly reliable. No false positive and only one false negative was encountered. An important advantage of this method is less sampling error than frozen sections because

numerous levels and facets of the biopsy can be readily assessed (Underwood J.C.E., 1981).

In 1934, Prof L.S.Dudgeon and Barrett published their continuing experiences with the wetfilm method as applied to surgically removed tissues and reported eight cases in which a diagnosis of malignancy was made from the wet film but not confirmed by histologic section, but the fate of these patients substantiated the malignant diagnosis. The authors concluded that the results of the method as applied to more than 1000 specimens compared favourably with examination of the corresponding paraffin sections.

Godwin the foremost advocate of this approach has stated that cytologic material obtained from surgical material submitted for rapid frozen sections is as readily diagnostic as the frozen section.

Elaustein P.A. and Silverberg S.G. (1977) emphasized the role of cytodiagnosis particularly when the specimen submitted is extremely small, crushed, soft, hermorrhagic, necrotic or mucoid.

Described by James Lignocaine Connolly et al (1988).

In 1989, S.Anuratha and V.Parthasarathy from BHEL Hospital, Tiruchirapalli, evaluated 50 cases of enlarged lymph nodes and concluded that imprint cytology is a rapid, easy, dependable method of diagnosis and in their study arrived at 94% diagnostic accuracy.

Imprint cytology has been evaluated laboratories in all parts of the world. Even though different techniques and stains have been deployed, the accuracy of diagnosis amounts to 97 to 98%.

Conclusions:-

Histology and cytology have proven not to be two competitive fields of human medicine, rather, they are able to improve the diagnostic result by complimenting one another.

FINE NEEDLE ASPIRATION CYTOLOGY

Rudolf Virchow who in 1855 stated the “every cell is derived from as cell” and that human disease processes were essentially diseases of the cells. Virchow is considered the protagonist of the concept of cellular pathology ‘or pathology based on the study of cells.

Investigation of tumour by means of a needle was carried out at St.barlolomew’s Hospital in 1833 for a case of abscess of the liver with hydatid cyst.

Subsequently Pravaz in 1853 and Gunter in 1882 used Pravaz syringe for transthoraic needle aspiration. Despite the diagnostic success, there appeared to be very little interest in this procedure during the ensuring 25 years. Attention was paid in tru-

cut and drill biopsies. The original authors used 18 gauge needle with air dried smears and require local anaesthesia. The scandinavians introduces the concept of ‘fine’ needle aspiration with a 23 guage needle, improved cytology fixation and staining technique. There was no need for local aneasthesia.

From time of time there have appeared warning about the danger of needle biopsy. Implementation metastasis in connection with percutaneous needle aspiration biopsy have occasionally been reported. Oschner and debaley (1942) condemned the procedure on the ground of possible spread of malignant and inflammatory disease. The authors did not record any proved cases of distant spread but described a case of implantation of tumour in the chest well. Grill and Hozard (1951) described a case in which silverman needle has been used to obtain a tissue cove from a papillary carcinoma of thyroid, subsequently cochin et al (1969), Ackerman and Wheat (1955) reported growth of malignant neoplasm along the needle track. However all these cases have been reported wide bore needles

were used. A search of literature has revealed no definite reports of local tumour extension caused by fine needle (18-20 gauge) aspiration biopsy (Sennir W.W. Zajicek, 1976).

Godwin defined fine needle aspiration as “the withdrawal of cells or small bits of tissue through a needle by means of a negative pressure”.

Normal Lymph Node Architecture:-

Lymph nodes are encapsulated structures composed predominantly of lymphoid tissue and supporting tissue situated along the lymphatic system that drains all regions and organs of the body. The fibrous capsule gives off trabeculae that penetrate into the parenchyma of the node and surrounds the peripheral or subcapsular sinus.

The subcapsular sinus continues into the cortical or trabcular sinus that traverse the paracortex and then become wider and more tortuous as they enter the medullary cords, where they become medullary sinus which converage at the hilum and exit as efferent lymphatics.

The lymph node consists of four structural and functional compartments.

1. The cortex with its well defined follicles proliferative sites of B-cells system.
2. The medually Zone most of plasma cells reside, secretory part of the B-cells system.
3. The paracortex, ill defined and lies between and extends deeper than the follicles and is the site where most of the T-cells are located.
4. The sinuses, which contain varying numbers of phagocytic histiocytes and are part of mononuclear phagocytic system.

Follicles are primary and secondary primary follicles are small, circumscribed collections of recirculating small lymphocytes within a supportive network of dentritic recticulum cells. The secondary follicles consist of a germinal centre that develops in the primary follicle after nodes are antigenically stimulated and the surrounding mantle zone, which is composed of small lymphocytes with characteristics

of primary follicle. At the outer edge of the mantle, zone is a poorly defined layer less densely packed cells called the marginal zone, with larger cells with more cytoplasm.

The germinal centres are characterized by a mixture of small and large cleared (centrocytes) and non-cleaved (centro blast) dentritic cells and macrophages. The medulla which lies deep to the paracortex, is divided into cords by the meduallry sinuses that converge upon the hilum. The medullary cords are the site where plasma cells are located and represent the secretory component of the B-cells system. Mast cells may also be present.

The para cortex is not well defined and is known as the T-cell zone, consisting of small T-cells with condensed chromatin and slightly irregular nuclear contours. This area is the major site of cellular immune response. Post capillary and high endothelial venules are located. The sinuses carry lymph from the afferent lymphatics. The sinuses are partially lined by endothelial cells and near the hilum by histocytes. They also

contain phagocytes. The histocytes are large cells with ample cytoplasm and large vesicular neclei. Varying numbers of small lymphocytes and occasional plasma cells may be found among the sinus histiocytes.

Cytology of a normal node shows a predominance of small lymphocytes as well as large lymphoid cells and scattered cells of other types.

Differential counts from normal lymph node imprints.

Lymphocytes – 67.8% to 90%

Polymorphs – 5.3% to 16.4 %

Monocytes – 0.2% to 7.4%

Lymphoblasts – 0.1% to 0.9%

Others include reticulum cells, mast cells monoblasts, plasmoblasts, plasma cells, neutrophils, basophils, eosionophils, proplasmocytes etc.

Abnormal lymph node cytology:

Criteria of malignancy in cytology, like those in histopathology are based in structure of individual cells as well as their altered inter relationship.

In cytology, interest is centered primarily on the morphologic characteristics of the individual cells, where as in tissue diagnosis, alteration is shifted in large measures to changes in the architectural pattern. Each method thus must be considered as complementing the other. Accurate identification of cells as to their type and origin is complicated by the extreme variation in both normal and abnormal cell types, by the presence of many intermediate forms and by their complete dissociation from their primary site. It is only through experience, persistent and systematic. Study over a period of years, that a more intimate knowledge of larger number of specific cell types can be acquired.

Lymphnode aspirates are studied with respect to

1.General criteria of malignancy in cytology

2. Cytology of specific lymph nodal pathology.

GENERAL CRITERIA OF MALIGNANCY IN CYTOLOGY

The genera criteria may be subdivided into

I. Criteria based on structural modification of cells and their nuclei.

II. Those based on changes in the interrelationships of cells as evidenced in cell clusters and tissue fragments.

III. Indirect criteria

I. Criteria based on structural modification of cells and their nuclei.

A. Changes in cells as a whole.

B. Nuclear changes

C. Cytoplasmic changes

A. Changes in cells as a whole

1. Enlargements of cells beyond their normal range.

2. Aberrance in the form of the cell aberrant or elongated cell forms having a normal nucleus. Should not be interpreted as malignant cells.

3. Degenerative or necrotic changes –necrosis is observed frequently in cases of malignant neoplasm and it is of great diagnostic value if present.

B. Nuclear changes

1. Disproportionate enlargements of the nuclei altering appreciably the normal nuclearcytoplasmic ratio.

2. Increase in chromatin content causing hyperchromasia overstaining may also give an impression of hyperchromasia and lead to a false evaluation of malignant cells.

3. Structural abnormalities such as an aberrant chromatin pattern, elongation, irregularity in outline, deep indentation and furrowing, lobulation and budding.

4. Enlarged nucleoli or increase in their number beyond normal variability. The term nucleolus is here used to designate both true nucleoli and karyosomes as their differentiation with our routine technique is not always possible.

5. Multinucleation when associated with nuclear atypia-multinucleation may result either from mitotic nuclear division without cytokinesis or from fusion of cells without fusion or nuclei.

6. Mitotic activity with abnormal mitotic figures. Abnormal mitotic figures may also be occasionally seen in malignant cells.

7. Marked thickening of the nuclear membrane. Thickening of the nuclear membrane by itself should not be stressed as a criteria for malignancy as it may be seen in chronic infection and other non malignant pathological conditions.

8. Degenerative changes such as abnormal vacuolation, fading or complete resorption of the nuclei. Vacuolation and fading of the nucleus may result from drying or irradiation.

C. Cytoplasmic changes:-

1. Changes reflected in staining reaction. Such changes may be brought to light by special techniques with the staining

procedures used in laboratories. Certain types of malignant cells may exhibit pronounced basophilia or acidophilia.

2. cytoplasmic inclusions such as pigment granules, leucocytes or cellular debris.

3. A typical vacuolation marked vaccuolation is frequently seen in adenocarcinoma cells although it occurs in other malignant cell forms. It is also observed in various types of non malignant cells under normal or pathological conditions.

II. Criteria based on the inter relationship of cells:-

1. Irregularity of pattern: It implies a lack of uniformity in the orientation of the cells and their nuclei.
2. Anisokaryosis and anisocytosis – It refers to marked variation in size of the nuclei and cells of the same type within a cluster, not to the variation found in single cells scattered throughout the smear. This is one of the most significant criteria of malignancy.
3. Lack of distinct cell boundaries.
4. Dense grouping and crowding of cells and nuclei.

5. Engulfment of one cell by another
6. Grouping of cells
7.

III. Indirect Criteria:-

1. Presence of blood
2. Excess of lymphocytes
3. Prominence of histiocytes
4. Polymorphonuclear leucocytes.

Comparison of features of benign and malignant cells

S.No.	Benign Features	Malignant Features
1.	Normal cell size	Increased cell size
2.	Good cell adhesion to one another	Loss of adhesion
3.	Uniformity of cells	Pleomorphism
4.	Rather coarse but regular nuclear chromatin	Fine, paler nuclear chromation often with prominent nucleoli
5.	Cellularity low	High
6.	Frequent stripped	Lack of stripped nuclei, lymphocyte response

Cytology of specific lymphnodal pathology

The cytology of specific lymphnodal pathology has its characteristic features by which it is possible to identify it. A brief description of these follows.

I. Metastatic carcinoma

It aspirates from metastatic nodes it is possible to identify malignant cells which are foreign to the lymph node. The cell of origin (primary) can be identified and the degree of differentiation determined.

Squamous cell carcinoma:-

Here, the smear shows large, irregular keratinished cells with abundant eosinophilic cytoplasm or relatively undifferentiated cells occurring in sheets with “windows” or spaces between individual cells and sharply outlined cell borders. Since the cells are clearly epithelial diagnosis obvious.

Lymphoepithlioma:-

The aspirate demonstrates very large undifferentiated tumour cells the frequently occur either singly or together in a

very loose arrangement, particularly in the air dried giemsa stained smears.

In the air dried smear, the pattern may suggest a malignant lymphoma, but, the alcohol fixed pappanicloau stained preparations more clearly demonstrate the aggregates of undifferentiated cells, with very prominent nucleoli and the surrounding lymphoidal elements that constitute the typical histological pattern of lymphoethelioma occasionally, they present as single cells. Many lymphocytes are also seen.

Small cell (OAT cell) carcinoma

Small cell carcinomas regardless of their site of origin show anaplastic, small, malignant cells that usually demonstrate good nuclear lining. These aspirates tend to be bloody and also contain numerous tomour cells. Neoplastic cells appear larger than one expects. Tissue specimen accurately reflect the same cytomorphologic features as those seen in the sputum of bronchial washings, from a case of out cell carcinoma of the lung.

Giant cell carcinoma:-

There is a large tumour giant cell that contains not only multiple nuclei but also many phagocytosed polymorphonuclear leukocytes. Surrounding of giant cell the undifferentiated tumour cells, some of these owing to their clear or vacuolated cytoplasm, might be mucicarmine positive, reflecting a basic adenocarcinoma pattern that has evolved into giant cell carcinoma.

Malignant melanoma:-

Malignant melanoma has several features other than pigmented cells that many identify it in metastatic sites. Double mirror image nuclei that face each other but do not touch are illustrated in several cells from both the giemsa stained and the pappanicalaou stained smears. Some melanomas also have large intranuclear inclusions. Of course the presence of pigment in melanoma cells is very helpful clue, as seen with romanovsky technique when the pigment stains dark bluish purple and pappanicalaou where it is brown.

Papillary carcinoma:-

Papillary fragments which demonstrate some cells with intranuclear inclusions seen in aspirates of enlarged cervical nodes give us a clue to the primary the thyroid gland.

II. Primary malignancies of the lymph nodes:-

Controversy surrounds the ability of aspiration cytology to pinpoint primary malignancy of the lymph nodes. However, features of findings in such cases have been described.

Non- Hodgkin's lymphoma:-

Clinically nodes of malignant lymphoma are larger than those secondarily involved. The smear patterns are monomorphic and reflect quiet accurately the cell type, whether it is a well differentiated or poorly differentiated lymphocyte, cleared or non cleared. The may-Gurnwald –Giemsa stain or other variants of the Romanovsky stain are quiet useful in identifying the specific cell type of malignant lymphoma. Obviously it is difficult to differentiates between the nodular and diffuse patterns of malignant lymphomas. The cells obtains by aspiration can be

used as surface makers and for other immumologic tests for specific cell classification.

Hodgkin's Lymphoma:-

The diagnosis of Hodgkin's disease from aspiration smears depends on the identification of Reedstenberg cells. These cells may demonstrate either the typical double mirror-image, overlapping nuclei or the type of pleomorphism that is seen particularly in the nodular sclerotic variety of Hodgkin's disease. An attempt has been made to subclassify Hodgkin's disease from the aspirate, but, this has usually been unsuccessful.

Chronic leukemia:-

Leukemia involvement of lymph nodes or organs can be confirmed easily by aspiration cytology. The battery of histochemical stains used for more precise classification of leukemia cells, particularly in the monocytes and granulocytic series can be applied here.

III Benign conditions:-

Lymphadenitis hyperplasia:-

Enlargement of lymph nodes on a reactive inflammatory basis accounts for the largest number of benign cases for which five needle aspiration biopsy is requested reactive hyper plasia must absolutely be differentiate from metastatic malignant tumour and malignant lymphoma. The smear pattern is one of a mixture of cell types, with lymphocytes of various degrees of differentiation and stimulation, plasma cells and enlarged histiocytic phagocytes some of which contain nuclear debris. The peculiar paleness appears to be effect of drying, which can be eliminated by the immediate fixation of the smear.

Infectious disease:-

While the emphasis in recent years has been on the aspiration diagnosis of tumours, infections diseases may also be specifically identified. The material is usable for culture. Several methods of diagnosising actinomycosis have been reported recently, including the application of immunofluorescence for the

specific identification of Actinomycosis Israeli. The “sulfer” granules are readily visible.

Granulomatous Inflammation :

Characterised by the presence of lymphocytes, plasma cells, and giant cells. Tuberculous lymphnode and sarcoidosis are characterized by the presence of granulomatous inflammation. Tuberculous inflammation is characterized by the presence of Langhan type of gaint cell and caseation necrosis.

METHODOLOGY AND PROTOCOL FOLLOWED

This is a prospective study period" extending from Jan 2007 to Sept 2008 in Govt Rajaji Hospital in our unit.

The patients presenting with cervical lymphadenopathy for whom open biopsy was planned were subjected to FNAC. At the time of open Biopsy imprint smear were taken and fixed.

Following final histopathology report the FNAC. Imprint cytology and HPE reports were compared and analysed.

Technique of FNAC

Materials:-

1. Disposable hypodermic needles of size 21 to 23 G with length of 1½ - 2 inches.
2. Disposable syringe
3. Glass slides
4. Disposable gloves
5. Cotton swabs with spirit
6. Koplin-jar for keeping slides in fixative
7. Isopropyl alcohol
8. Hematoxylin and eosin stains

Technique for aspiration:-

a) Introduction of needle:-

- Skin over the swelling is cleaned.
- The syringe with needle is held in dominant hand while the other hand fix the swelling.
- The needle is introduced centrally in the swelling.

b) Maintaining negative pressure:-

- Once the needle is introduced a negative pressure is created by retracting the plunger.
- This negative pressure is maintained till the required amount of material is seen in the hub of the needle.
- The function of negative pressure
- Not to tear cells from tissues but to hold the tissue against the sharp cutting edge of the needle.
- The softer tissue components that protrude over the edge are cut and accumulate in the lumen of the needle as the needle is advanced.

Needle manovere:-

- To obtain adequate specimen the needle must be manovered within circumscribed area in different directions.
- If blood is observed in the syringe the procedure is stopped and another specimen is procured.
- Release of negative pressure and withdrawal.
- The negative pressure is released prior to the withdrawal of the needle to avoid centrifugal force expelling the specimen and possible tumor seedling. Failure to comply with this step may result is air rushing up the needle and loss of specimen into the body of the syringe. Then the syringe is gently withdrawn. Firm pressure is applied to the aspiration site to avoid hemorrhage.

e. Transferring the aspirate on to the slides.

The needle is removed from the syringe the plunger is retracted completely, the needle is reattached and plunger is forcibly pushed to express the droplets.

Air Reservoir Technique:-

In this technique 5ml of air is drawn into the syringe prior to the introduction of the needle into the lesion. After introducing the needle the syringe is pulled again to the find volume and the manover is carried over the described already.

After aspiration the plunger is released slowly. The fall in suction allows the plunger to return to original JML mark. This technique eliminates the need for removed of syringe from the needle.

Non-aspiration technique:-

Here syringe and negative pressure is not used only needle alone is introduced into different direction.

It gives a clear and less hemorrhagic material and allows smaller lesion to be sampled with less difficulty.

Smearing:-

Aspirate may be dry or wet type.

Dry aspirate contains numerous cells suspended in small amount of tissue fluid and creamy in consistently.

The wet aspirate contains less number of cells suspended in fluid or blood.

Dry aspirate:-

Dry aspirate is smeared with the help of 0.4mm cover slip by exerting a light pressure to obtain thin evenly spread smear.

Too much pressure produces artifact.

It is better to make

Thicker smear than thinner one

The smear is fixed before drying.

Wet aspirate:-

The cover slip is moved to the middle of the slide while holding it at an angle with slide. This leaves most of the fluid behind. While the cells follow the cover slip like a buffy coat.

The concentrated cells are smeared now with the flat of the coverslips as for dry aspirate.

Fixation and staining:-

Fixation does not require more than a few minutes but a minimum of fifteen minutes is advisable for proper adhesion of the smear to the slide. It is necessary that smears be kept in the alcohol either solution over a long period of time. However this is not recommended as a general rule, as it may result in an alteration in the staining reaction of the cells.

A number of fixatives are used in cytology. The common ones are modifications of 95 percentage ethyl alcohol. 95 percentage of ethyl alcohol can be used on its own with satisfactory results. But, the addition of 3 % glacial acetic acid increased the nucleoprotein fixing properties. This is the standard fixative and gives excellent nuclear and cytoplasmic morphology.

Smears which are be mailed to a laboratory for staining should be fixed in alcohol ether for atleast on hour. If ether is not available 95 percentage ethyl alcohol alone can be used.

Methyl or even isopropyl alcohol, though less desirable can be used, as substitutes if chemically pure.

Staining procedures:-

Smears can be stained by pappanicolaou technique or by standard hematoxycin and eosin methods. The basic constitutent of both stains is harris hematoxylin.

Harris hematoxylin:-

This is an alum hematoxylin which is chemically ripened with mercuric oxide. It is a useful general purpose hematoxylin and gives particularly clear staining and for this reason has been used in diagnostic cytology with an eosin as counter stain.

It routine histological use Harris Hematoxylin is used regressively but in cytology it may be used as a progressive stain.

This stain is prepared as follows:-

Hematoxylin = 2.5gms

Absolute alcohol	=	25cc
Potassium alum	=	50 gms
Mercuric oxide	=	1.2 gms
Glacial acetic acid	=	20 cc
Distilled water	=	500cc

The hematoxylin is dissolved in absolute alcohol and is then added to alum which has previously been dissolved in warm distilled water in a two litre flask. Mercuric oxide is added. The stain is rapidly cooled by plunging the flask into cold water or into a sink containing ice. When the solution is cold the acetic acid is optional but its inclusion gives more precise and selective staining of the nuclei.

As with most of the chemically ripened alum hematoxylin the quality of the nuclear staining begins to deteriorate in a few months. The deterioration is marked by the formation of a precipitate in the stored solution. At this stage the stain should be filtered before use and the staining time may need to be increased. For the best results it is wise to prepare a fresh batch of

stain every month although this may not be economical unless only small quantities are prepared each time.

Eosin:-

Eosin is the most suitable stain to be combined with an alum hematoxylin to demonstrate the general histological architecture of a tissue. Its particular value is its ability, with proper differentiation, to distinguish between the cytoplasm of different types of cells. The eosins are Xantine dyes and the following types are easily obtainable commercially.

1. Eosin Y (Eosin yellowish, eosin soluble)
2. Ethyl eosin S (Eosin alcohol solution eosin)
3. Eosin B (Eosin bluish, erythrosine B)

Of this Eosin Y is the most widely used and despite its synonym, it is also satisfactorily soluble in alcohol. As a cytoplasmic stain it is usually used as 0.5% to 1% solution in distilled water with a crystal of thymol added to inhibit the growth of fungi addition of a little acetic acid is said to sharp the

staining. Differentiation of the eosin staining during sufficient tap water washing and a little further differentiation occurs during the dehydration through the alcohol. The intensity of eosin staining and degree of differentiation required is largely a matter of the individual pathologists's choice.

Pappanicolou Technique:-

When available this is the method of choice in cytology because it gives a dependable nuclear morphology with clear translucent demonstration of the cytoplasm.

For this the following stains are to be prepared.

1. Harris Hematoxylin
2. O.G. 6
3. EA 50

Method:-

1. Rinse the side in 95% alcohol
2. Rinse in 70% alcohol
3. Rinse in distilled water

4. Stain in Harris Hematoxylin for 5 to 30 seconds

5. Rinse in distilled water

6. Rinse in 95% alcohol

7. Treat with 0.1% ammonia in 95% alcohol for one minute.

8. Rinse in 95% alcohol

9. Stain in 0.5.6 for 90 seconds

10. Rinse in 95% alcohol

11. Stain in E.A. 50 for 90 seconds

12. Rinse in 95% alcohol twice

13. Rinse in absolute twice

14. Rinse in xylol twice

Results:-

Nuclei : Blue to black

Cytoplasm : Pink to green

R.B.C. : Orange to red

Fibrin : Deep pink

Hematoxylin and eosin:-

Although this technique cannot match that of pappanicolou for clarity and detail, many cytopathologist's prefer this method because they are already familiar with Hematoxylin –Eosin staining on histological sections.

Procedure for Hematoxylin –Eosin Staining:-

1. Take the smear to water
2. Stain with Harris Hematoxylin for 3-10 minutes
3. Wash in running tap water
4. Differentiation in acid alcohol
5. Wash briefly in water
6. Dip several times in Lithium carbonate or ammonia water, the smear will change to blue colour.
7. Wash in water.
8. Counter-stain with eosin for a few seconds to a minute
9. Place in 70% alcohol
10. Place in 95% alcohol

11.Place in absolute alcohol

12.Clear in xylin

Technique of Imprint cytology:-

In surgical specimen before formalin fixation multiple cur sections are made. They are blotched dry of blood. The cut surfaces were gently touched on the glass slides. Fixation is done by using Isopropyl alcohol 95% and are transported to pathologist.

The slides are passed through descending grades of alcohol i.e. 80% 75% 50% to slides then covered with haermotoxylin stain for 3 min rinsed in tap water for 1 to 2 min. Then treated with acid alcohol for few seconds till excess stain is cleared. Then slides are covered with 1% eosin for 1 minute, rinsed in tap water allowed to dry and examined under microscope.

OBSERVATION AND RESULTS

Totally seventy patients with cervical lymphnodes were taken up for this study irrespective of age and sex. These includes patient attended surgical OP and referred from medical side for lymphnode biopsy.

Those who gave consent for open surgery only taken up for study. FNAC was taken first and the patient was instructed to return for open biopsy at a later date. During open biopsy the specimen was cut and the slides are touched and sent for HPE with full clinical details.

Out of the 70 lymphnodes examined 34 were benign nodes 16 were reactive nodes and 18 were tuberculosis nodes.

FNAC picked up 11 tuberculosis nodes and the 7 reported as reactive nodes, imprint cytology could pickup only 10 cases.

Out of the 36 malignant cases 32 cases were detected by imprint and 4 were acellular. 29 cases were detected by FNAC.

Out of the 36 malignant reports 9 were due to secondaries from papillary carcinoma thyroid, 18 were due to infiltration with squmous cell carcinoma. 3 were due to adenocarcinoma. 6 cases were reported as Hodgkins lymphoma. All the 6 cases were reported from medical side.

Disease	Biopsy proved	FNAC			Imprint cytology		
		True positive	False positive	False negative	True positive	False positive	False negative
Metastatic deposits	30	26	0	4	27	0	3
Lymphoma	6	3	0	3	5	0	1
Tuberculosis	18	11	0	7	10	0	8
Reactive hyperplasia	8	5	5	3	6	4	2
Non specific	8	4	6	4	5	5	3
Acellular		10			8		

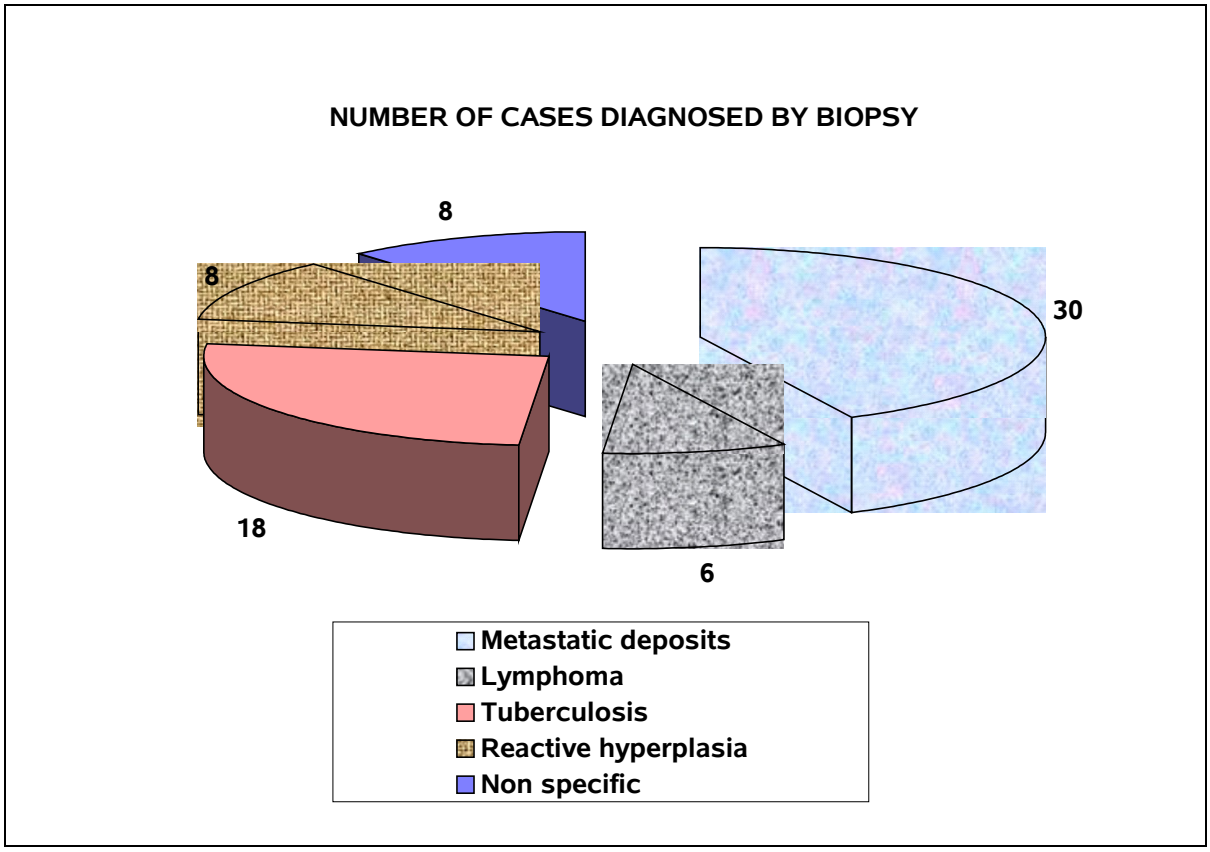
Depending on the results obtained sensitivity, specificity and positive predictive value are calculated.

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + false negative}} \times 100$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative + false positive}} \times 100$$

$$\text{Positive predictive value} = \frac{\text{True positive}}{\text{True positive + false positive}} \times 100$$

Accuracy of the test is the reflection of the ability of the test to identify all positive cases (sensitivity).



Disease	Sensitivity		Specificity		Positive predictive value	
	FNAC	Imprint	FNAC	Imprint	FNAC	Imprint
Secondary Deposit	86.7	90	100	100	100	100
Lymphoma	50	83.3	100	100	100	100
Tuberculosis	61.1	55.6	100	100	100	100

Out of the 6 lymphoma cases all were reported as Hodgkins lymphoma by histophathology. 3 were reported as lymphoma and 2 were reported as suggestive of lymphoma by imprint, whereas only 3 cases were picked up by FNAC.

The diagnostic accuracy for tuberculosis adenitis was 61% and for secondary malignant deposit 86.7% for FNAC. For imprint cytology the figures were 55.6%, 90% respectively.

DISCUSSION

In lymph node lesions detection of primary lymphoma by Imprint 83.3% accuracy though the exact type could not be made out. By FNAC it is 50%. The secondary deposit detection by imprint and FNAC are 90% and 86.7%. In tuberulosis nodes out of the 18 cases detected by HPE 11 cases were picked up by FNAC and 10 cases by Imprint. This may be due to abundant caseous material with few epitheloid cells which makes the imprinting and interpretation difficult.

In lymph node lesions FNAC and Imprint are useful in diagnosing metastatic neoplasm rendering primary diagnosis of lymphoma in documenting evolution of low grade lymphoma to high grade, in diagnosing non specific and certain specific lymphadenitis.

Benign lymphoid hyperplasia can mimic malignant lymphoma and vice versa (eg) Benign proliferation of large transformed lymphocytes may be misinterpreted as malignant

lymphoma and conversely low grade NHL resemble reactive lymphnode hyperplasia. In cytology low grade NHL can't be distinguished from benign lymphonode. 90% of high grade NHL are identified correctly. In Hodgkins lymphoma diagnosis can be made but sub typing may not be feasible by FNAC but can be made to some extent by Imprint. RS cells are makers. In lymphocyte predominant type typical RS cells are infrequent. In nodular sclerosis cell yield is poor. When Imprint is combined with Immunohistochemisty the exact typing of lymphoma and metastatic disease can be diagnosed more accurately.

In metastatic disease FNAC and Imprint scores well in accuracy. In 1952 Dearing reported from Newcastle the use of smear to determine metastasis in lymphnodes by scrapings from cut node fixing in carnolys solution and rapid staining with papenicolaru stain. In 219 smears 98.6% correct diagnosis were made. In our study the diagnostic accuracy amounts to 86.7%.

A study by Motomura et al (2000) on intraoperative lymphnode examination by Imprint cytology and frozen section

has concluded that the sensitivity of frozen section was almost equivalent to that of Imprint cytology.

In cytology, determination of malignancy is more important than benign lesions. In this aspect there were no false positives either in FNAC or Imprint, cytology. In lymph node lesions there was one false negative in lymphoma in imprint cytology and three false negative in FNAC. For secondary deposits there were seven false negative reporting in FNAC and Imprint cytology.

REVIEW OF LITERATURE

Comparison of Results with Other studies

Diagnostic Accuracy rate of FNAC

Lymphoma :

1.	Das et al (Acta cytol, 1990)	88%
2.	Gupta et al (Ind. J. of Path 1977)	88%
3.	Russel et al (Aus J. Med. 1983)	90%
4.	Our study	50%

Low accuracy rate in our study is due to less number of cases studied.

Secondary Deposit

1.	Kline and Heal Jama 1976	96%
2.	Piscioli et al Cancer 56, 1985	93%
3.	Zedelza et al Cancer, 1976	96%
4.	Our study	86.6 %

Results in our study almost correlates with other studies

Diagnostic Accuracy of Imprint cytology

Lymphoma

1. Diagnostic cytopath Greece - 91%
2. Our study - 83.3 %

Secondary Deposit :

1. Actecytol 1982 - 89.9 %
2. Our study - 90%

CONCLUSION

1. Both FNAC and Imprint cytology have definite role in the diagnosis of cervical adenopathy.
2. The diagnostic accuracy for Imprint cytology amount to 61.8% for benign and 88% for malignant infiltration of lymphnode. When FNAC and Imprint were taken together the diagnostic accuracy is 88.9% for malignant and 62.8% for benign for lymph node lesions.
3. There were no false positive for malignancy either in FNAC or imprint cytology though there were a few false negative reportings. The false negative reporting were higher in FNAC than Imprint.
4. In metastatic involvement both FNAC and Imprint cytology were very sensitive and the pathologist can able to pinpoint the primary in some cases. The degree of differentiation can also be made out in both methods.

5. Intra operatively imprint cytology is done whenever immediate results are needed and with this regard imprint cytology is even superior to frozen section as for as lymphnode is concerned. (RD collin Arch. Path Lab Med 1985).
6. Imprint cytology when taken properly and when combined with immunological technique can diagnose the subtype of lymphoma.

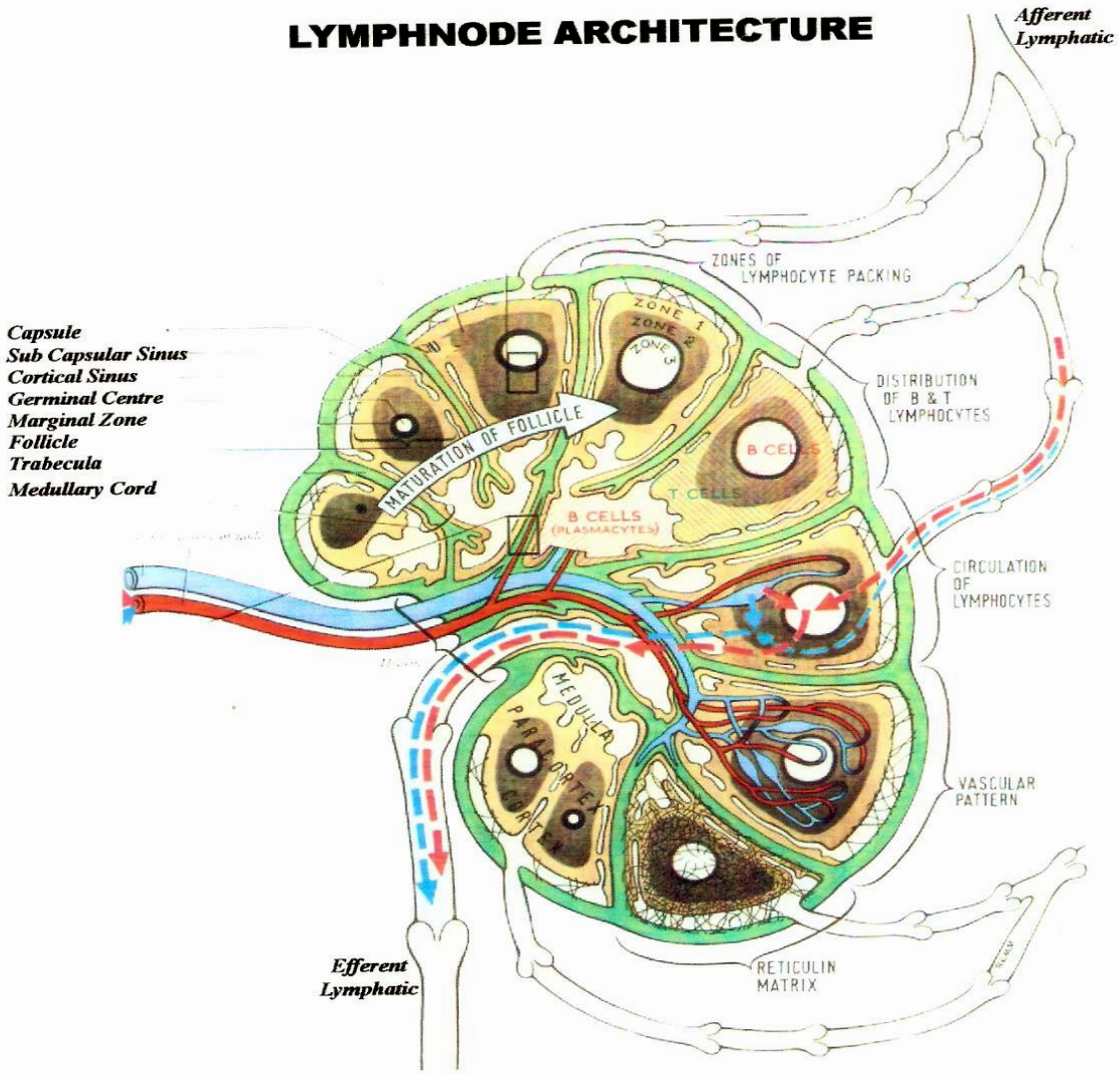
To conclude FNAC and Imprint cytology are not competitive but complimentary to each other in diagnosing dose of lymphonode. Whenever required method may supplement the other for rapid and increased accuracy of diagnosis.

BIBLIOGRAPHY

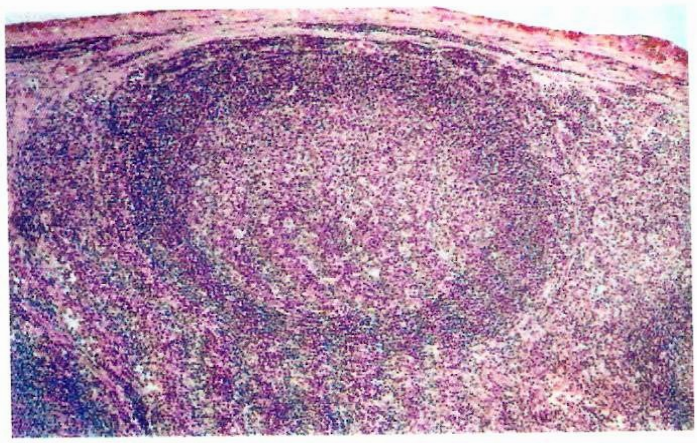
1. Koss diagnostic cytology 5th edition
2. Bibbo comprehensive cytopathology 2nd edition
3. Stenberg diagnostic surgical pathology 4th edition
4. Manual of histological staining method Lee. G. Luna
5. Pathology for Surgeon's Anderson
6. Moore RD and Reagon J.W. cellular, study of lymph node imprints.
7. Manual and atics of FNAC – R. Orell / Sterret, wel ters, whitaken – 3rd edition.
8. Pathological basis of disease – Robbins.
9. Boyd Text book of pathology – 8th edition
10. Text book of Surgical pathology – Sir Charles Illingworth 12th edition.
11. Banforth J, Osborn G.R. : Diagnosis from cells. J. Clinical Pathology 11: 475- 482, 1958.
12. Bland-Sutton J, : Tumours innocent and malignant, ed. 7 London, Cassell, 1922, cited by Banforth.

13. Compels B.M., Pike C.P. : FNAC, Lancet II : 429, 1979.

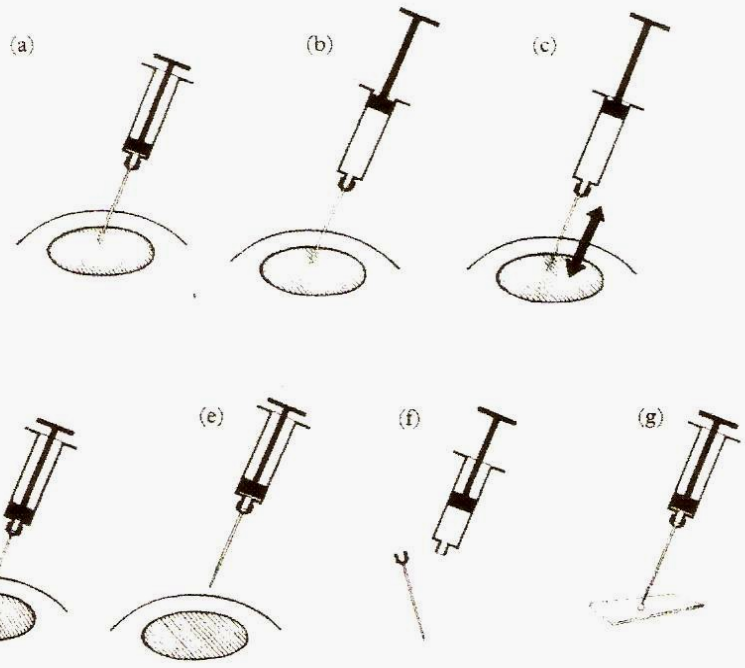
LYMPHNODE ARCHITECTURE



LYMPHNODE HISTOLOGY



FNAC TECHNIQUE

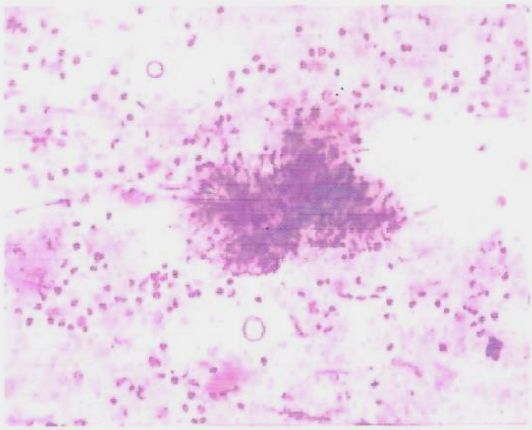


a) Needle Positioning
b) Applying Negative Pressure
c) Needle Manouvere
d) Release of Negative Pressure
e) Needle withdrawal
f) Needle detached, air drawn in
g) Blowing the aspirate on to slide

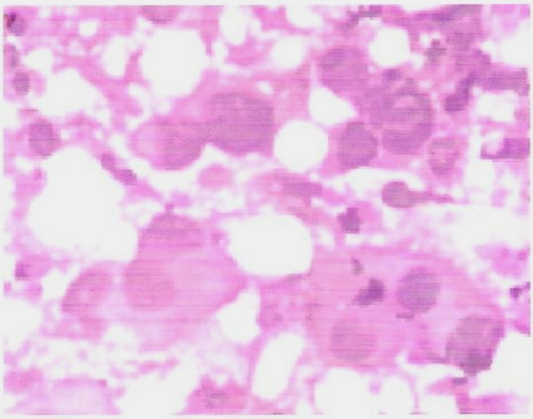
SMEARING TECHNIQUE FOR DRY ASPIRATE

SMEARING TECHNIQUE FOR WET ASPIRATE

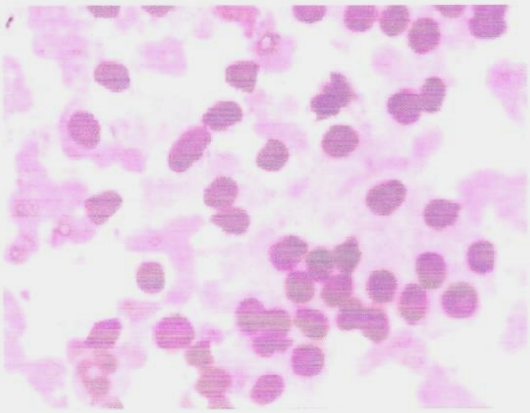
GRAUNULOMATOUS ADENITIS - IMPRINT



SECONDARY DEPOSITS - IMPRINT



LYMPHOMA - IMPRINT



MASTER CHART

S.No.	Name	Age / Sex	IP No.	Clinical Diagnosis	FNAC	Imprint	Biopsy
1.	Lakshmi	43/F	345502	Ca Thyroid	Pap.Carcinoma	Pap.Carcinoma	Pap.Carcinoma
2.	Kanchana	26/F	413512	Lymphoma	Lymphoma	Lymphoma	Lymphoma
3.	Malarvizhi	27/F	24410	TB adenitis	TB adenitis	Acellular	TB adenitis
4.	Mahalingam	75/M	236097	Lymphoma	RH	Lymphoma	Lymphoma
5.	Vairavan	85/M	419454	Secondaries neck	Secondaries neck	RH	Secondaries neck
6.	Meenakshi	60/F	51014	Secondaries neck	RH	RH	RH
7.	Muthu	39/M	33663	TB adenitis	TB adenitis	TB adenitis	TB adenitis
8.	Kumar	24/M	15788	Cervical adenopathy	Acellular	Acellular	TB adenitis
9.	Jeya	35/F	15193	Cervical adenopathy	Acellular	NSA	RH

10.	Murugaiyah	55/M	412466	Cervical adenitis	Acellular	NSA	RH
11.	Ismail	37/M	422184	Cervical adenitis	RH	RH	RH
12.	Aruldoss	67/M	32020	Secondaries neck	RH	Acellular	RH
13.	Muthammal	45/F	58271	Ca tongue	Sec. INFL	Sec. INFL	Sec. INFL
14.	Pitchaithai	50/F	38437	Tuberculous adenitis	TB adenitis	TB adenitis	TB adenitis
15.	Muthiah	55/M	53864	Ca thyroid	Pap.Carcinoma	Pap.Carcinoma	Pap.Carcinoma
16.	Muthulakshmi	26/F	26875	Cervical adenitis	Acellular	RH	TB adenitis

17.	Meenakshi	61/F	66694	Secondaries neck	Secondaries neck	Secondaries neck	Secondaries neck
18.	Mani	37/M	369270	Secondaries neck	Secondaries neck	Secondaries neck	Secondaries neck
19.	Ramesh	21/M	465718	TB adenitis	Acellular	Acellular	TB adenitis
20.	Ganesan	45/M	419382	TB adenitis	NSA	NSA	NSA
21.	Janaki	51/F	434027	Ca Bronchus	Acellular	NSA	Sec. INFL
22.	Pankajam	56/F	435679	Ca Breast	Sec. INFL	Sec. INFL	Sec. INFL
23	Muthumari	50/F	76356	Ca Stomach	RH	RH	RH

24.	Sathya	16/F	43794	TB adenitis	TB adenitis	TB adenitis	TB adenitis
25.	Palanikumar	21/M	452835	TB adenitis	Acellular	NSA	TB adenitis
26.	Kannan	31/M	412619	Ca thyroid	Pap. Carcinoma	Pap. Carcinoma	Pap. Carcinoma
27.	Jothi	80/M	443210	Cervical Adenitis	TB adenitis	TB adenitis	TB adenitis
28.	Nisha	19/F	443195	TB adenitis	NSA	NSA	TB adenitis
29.	Pitchammal	50/F	409866	Ca Lip	RH	Sec INFL	Sec INFL
30	Ponnammal	36/F	413552	TB adenitis	Acellular	NSA	TB adenitis

31.	Selvakani	26/F	412378	TB adenitis	TB adenitis	TB adenitis	TB adenitis
32.	Ilayaraja	18/M	1022	Lymphoma	Lymphoma	Lymphoma	Lymphoma
33.	Karuppaiya	73/M	472154	Ca Lung	Sec INFL	Sec INFL	Sec INFL
34.	Gurusamy	60/M	435938	Ca Bronchus	Sec INFL	Sec INFL	Sec INFL
35.	Meena	30/F	430210	TB adenitis	TB adenitis	TB adenitis	TB adenitis
36.	Karuppayee	53/F	3642	Ca Stomach	Sec INFL	Sec INFL	Sec INFL
37	Muthukali	68/M	2147	Cervical	NSA	Acellular	NSA

				adenopathy			
38.	Sathyavathi	42/F	50670	Ca thyroid	Pap.Carcinoma	Pap.Carcinoma	Pap.Carcinoma
39.	Thangathai	55/F	165903	Ca cheek	Sec INFL	Sec INFL	Sec INFL
40.	Nathan	23/M	31423	Lymphoma	RH	Lymphoma	Lymphoma
41.	Athirammal	29/F	458166	TB Adenitis	RH	RH	RH
42.	Jothilakshmi	21/F	242812	TB Adenitis	TB Adenitis	TB Adenitis	TB Adenitis
43.	Selvaraj	23/M	56608	Ca Thyroid	Pap.Carcinoma	Pap.Carcinoma	Pap.Carcinoma
44	Andichiammal	55/F	61425	Cervical	Acellular	Acellular	NSA

				adenopathy			
45.	Adaikan	65/M	62689	Ca tongue	Sec INFL	Sec INFL	Sec INFL
46.	Ilayaraja	15/M	777339	TB Adenitis	TB Adenitis	TB Adenitis	TB Adenitis
47.	Ramuthai	60/F	71253	Ca cheek	Sec INFL	Sec INFL	Sec INFL
48.	Kannan	15/M	138377	TB Adenitis	TB Adenitis	TB Adenitis	TB Adenitis
49.	Muthukaruppan	60/M	80829	Ca stomach	Sec INFL	RH	Sec INFL
50.	Sasithabanu	15/F	84700	TB Adenitis	Acellular	RH	TB Adenitis
51	Vairamoorthy	40/M	81193	Ca thyroid	Pap.Carcinoma	Pap.Carcinoma	Pap.Carcinoma

52.	Mariammal	42/F	455564	Ca thyroid	Pap.Carcinoma	Pap.Carcinoma	Pap.Carcinoma
53.	Palaniammal	23/F	84738	Ca thyroid	Pap.Carcinoma	Pap.Carcinoma	Pap.Carcinoma
54.	Muthumani	20/M	12008	TB Adenitis	RH	RH	RH
55.	Karuppasamy	60/M	90401	Ca stomach	Sec INFL	Sec INFL	Sec INFL
56.	Murugan	30/M	90935	Cervical adenitis	NSA	NSA	NSA
57.	Jothimani	70/F	464174	Ca cheek	RH	Sec INFL	Sec INFL
58	Pandian	80/M	89372	Ca tongue	Sec INFL	Sec INFL	Sec INFL

59.	Subban	68/M	89971	Ca hypopharynx	Sec INFL	Sec INFL	Sec INFL
60.	Alagumeena	18/F	90102	TB Adenitis	TB Adenitis	TB Adenitis	TB Adenitis
61.	Muthuselvi	28/F	89340	Ca thyroid	Pap.Carcinoma	Pap.Carcinoma	Pap.Carcinoma
62.	Arumugam	40/M	19230	Lymphoma	Lymphoma	Lymphoma	Lymphoma
63.	Ramasamy	64/M	72920	Ca larynx	Acellular	Sec INFL	Sec INFL
64.	Raju	38/M	22418	Lymphoma	RH	RH	Lymphoma
65	Ganapathi	25/M	33418	Cervical Adenitis	NSA	NSA	NSA

66.	Ramalakshmi	45/F	35423	Secondary neck	Sec INFL	Sec INFL	Sec INFL
67.	Narayanan	55/M	34400	Ca stomach	Sec INFL	Acellular	Sec INFL
68.	Arputhamani	29/F	1123	TB Adenitis	NSA	NSA	NSA
69.	Deivenathen	31/M	2341	Cervical adenitis	NSA	NSA	NSA
70.	Nagaraj	23/M	3262	Cervical adenitis	Acellular	Acellular	NSA